Quantitative Diffusion Tensor Imaging of White Matter Microstructure in Dog Brain at 7T †

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Abstract: The purpose of this study is to develop a noninvasive quantitative imaging scheme to assess the functional microanatomy of dog brain using high resolution diffusion tensor imaging (DTI) at 7-Tesla (T) magnetic resonance imaging (MRI) scanner. Diffusion weighted images (DWIs) along 25 diffusion-encoding directions, combined with T2 weighted images, were acquired using the standard spin-echo diffusion encoding scheme to determine the diffusion tensor matrix. Three diffusion indices: fractional anisotropy (FA), Trace/3 apparent diffusion coefficient (ADC), and volume ratio (VR) were measured within seven representative regions of interest (ROIs) from internal capsule, corpus callosum, caudate nucleus, hippocampus, thalamus, fornix, and cerebral cortex. Experimental results show much higher scalar contrast and more accurate fiber structures based on the FA weighted color map and fiber orientation map, indicating this high angular DTI technique at 7T is an accurate scheme to map the microstructures in fixed dog brains, which is also applicable for studying the functional microanatomy of other animal models.

Keywords: Magnetic resonance imaging (MRI), diffusion tensor imaging (DTI), dog brain, white matter, microanatomy.

1. INTRODUCTION

Magnetic Resonance Diffusion Tensor Imaging (MR-DTI) [1, 2] is a novel method to measure water diffusion anisotropy in biological tissue. In some tissues, for example cerebrospinal fluid (CSF), Brownian motion leads water molecules to diffuse freely in any direction. In other tissue, such as white matter (WM), the highly-organized fibrous structure restricts the diffusion of water, resulting in the mobility of water along the fiber orientation is much easier than across it. DTI is an imaging modality that can measure this directional or anisotropic diffusion, thus it is particularly useful for studying functional microanatomy in normal or diseased human brains [3-6].

DTI technique has also been applied to image animal brains *in vivo* and *ex vivo* [7-9]. Previous work [10] shows consistent results between diffusion anisotropy of *in vivo* and *ex vivo* formalin-fixed mouse brains. This offers a new opportunity to study the brain microstructure with *ex vivo* DTI, as it avoids motion artifacts and allows for longer imaging time.

Our work and others have shown that multiple-angular diffusion encoding schemes provide much more accurate measurement and computation of diffusion anisotropy maps [11-13]. A drawback for such DTI experiments is the long acquisition time. Using formalin-fixed whole brain samples overcomes this problem, resulting in a valuable investigative tool which allows high spatial resolution and improved signal-to-noise ratio (SNR) data achieved through the higher field strength [14].

In this study we performed a high angular DTI on the fixed dog brains at 7T. The diffusion weighted images along 25 diffusion-encoding directions, including T2 weighted images, were acquired using the standard spin-echo diffusion encoding scheme in order to estimate the 3×3 diffusion tensor matrix. Three diffusion indices: FA, Trace/3 ADC, and VR were measured separately within seven ROIs: internal capsule, corpus callosum, caudate nucleus, hippocampus, thalamus, fornix, and cerebral cortex. Experimental results showed much higher scalar contrast and more accurate fiber structures based on the FA weighted color map and fiber orientation map, which prove that better diffusion tensor model can been estimated from this scheme.

Although animal brains have been investigated extensively with DTI technique [7, 15-17], to our knowledge, systematic and quantitative DTI examination for dog brain has not been reported previously. The goal of this paper is to provide an accurate and quantitative analysis of the microstructures in dog brain, the methodology can also be used to study the functional microanatomy of other animal models.

2. MATERIALS AND METHODOLOGY

2.1. Diffusion Tensor Imaging

Diffusion Tensor Imaging is a specific MRI modality for imaging the fibrous structure of tissue in the body using the diffusivity of water. By acquiring diffusion-weighted images in at least six non-collinear directions, it is possible to estimate a 3×3 symmetric matrix (i.e. diffusion tensor $\mathbf{D}$)

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using a relationship between the measured echo attenuation in each voxel and the applied magnetic field gradient sequence [18, 19]:

$$\ln\left(S = \frac{A(\mathbf{b})}{A(\mathbf{b}=\mathbf{0})}\right) = -\gamma^2\delta^2(\Delta - \delta/3)\sum_{i,j} G_i D_{ij} G_j = -\sum_{i=1}^{3}\sum_{j=1}^{3} b_{ij} D_{ij}$$

$$= -\left(b_{xx}D_{xx} + 2b_{xy}D_{xy} + 2b_{xz}D_{xz} + b_{yy}D_{yy} + 2b_{yz}D_{yz} + b_{zz}D_{zz}\right), \quad (1)$$

Here γ is the the gyromagnetic ratio of protons (42.57 MHz/Tesla), δ is the diffusion gradient duration and Δ is the diffusion gradient separation. In MR-DTI a symmetric b-matrix, **b**, summarizes the attenuating effect of all gradient waveforms applied in all three directions, x, y and z; and b_{ij} represents the elements of the b-matrix of row *i* and column *j*. In general, for *m* diffusion-encoding directions, we solve the system of equations

$$\begin{bmatrix} \ln S^1 \\ \vdots \\ \ln S^m \end{bmatrix} = \begin{bmatrix} -b_{xx}^1 & -2b_{xy}^1 & -2b_{xz}^1 & -b_{yy}^1 & -2b_{yz}^1 & -b_{zz}^1 \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ -b_{xx}^m & -2b_{xy}^m & -2b_{xz}^m & -b_{yy}^m & -2b_{yz}^m & -b_{zz}^m \end{bmatrix} \times \begin{bmatrix} D_{xx} \\ D_{xy} \\ D_{xz} \\ D_{yy} \\ D_{yz} \\ D_{zz} \end{bmatrix}, \quad (2)$$

or in corresponding matrix form

$$\ln(\mathbf{S}) = \mathbf{B} \times \mathbf{d}, \quad (3)$$

where the superscript *1, …, m* in equation (2) are direction indices. **d** can be estimated as

$$\mathbf{d} = \left(\mathbf{B}^T\mathbf{B}\right)^{-1}\mathbf{B}^T \ln(\mathbf{S}) \quad (4)$$

through the multivariate linear regression of equation (2), which further constitutes the 3×3 symmetric tensor matrix

$$\mathbf{D} = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{xy} & D_{yy} & D_{yz} \\ D_{xz} & D_{yz} & D_{zz} \end{bmatrix}. \quad (5)$$

By diagonalizing the tensor **D**, one can get its three eigenvalues (λ_1, λ_2, and λ_3) and the associated three eigenvectors ($\mathbf{v}_1, \mathbf{v}_2$, and $\mathbf{v}_3$) for each voxel. The eigenvector corresponding to the largest eigenvalue is conventionally assumed to point along the direction of a fiber bundle. The commonly used diffusion indices include:

Fractional Anisotropy

$$FA = \sqrt{\frac{3}{2}}\sqrt{\frac{(\lambda_1 - \bar{\lambda})^2 + (\lambda_2 - \bar{\lambda})^2 + (\lambda_3 - \bar{\lambda})^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}},$$

$$(\bar{\lambda} = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}), \quad (6)$$

which is the most general approach to describe the degree of anisotropy with the value ranging from 0 (isotropic diffusion) to 1 (maximum anisotropic diffusion).

Trace/3 Apparent Diffusion Coefficient

$$ADC = \frac{D_{xx} + D_{yy} + D_{zz}}{3} \quad (7)$$

is usually used to characterize the overall displacement of the water molecules. It is not susceptible to diffusion gradient applied directions, therefore the ADC contrast is insensitive to the anisotropy effect.

Volume Ratio

$$VR = \frac{\lambda_1 \cdot \lambda_2 \cdot \lambda_3}{\bar{\lambda}^3}, \quad (8)$$

which represents the ratio of the ellipsoid volume (with three semi-axis length λ_1, λ_2, and λ_3) to the volume of a sphere of radius $\bar{\lambda}$. Unlike FA, the range of VR from 0 to 1 denotes the diffusion from maximum anisotropic to isotropic.

2.2. Experimental Samples and Image Acquisition

Five whole dog brains were collected and provided by the Small Animal Clinical Services at the VA-MD Regional College of Veterinary Medicine at Virginia Tech. The animals died of natural causes, and their brains were removed and immersed in 10% neutral buffered formalin for fixation.

MRI experiments were performed on a 7T Bruker Biospin magnet (Bruker, Ettlingen, Germany), with a gradient coil capable of generating maximum gradient amplitude of 400mT/m. A 7.5cm ID volume coil was used for RF transmitting and receiving. A conventional, multislice, spin-echo imaging sequence, modified by adding a Stejskal-Tanner diffusion-sensitizing gradient pair, was employed to acquire the required series of diffusion-weighted images. DWIs along 25 diffusion-encoding directions, including T2 weighted images, were acquired using the standard spin-echo diffusion encoding scheme, with the following parameters: Repetition Time (TR) = 1000ms, Echo Time (TE) = 36 ms, b = 2500s/mm^2, diffusion gradient duration (δ) = 6.0ms, diffusion gradient separation (Δ) = 8.21ms. The slice geometry parameters were: slice thickness = 0.6mm, image matrix size = 256×256, FOV = 7.0×7.0 cm^2, resulting in the voxel size of 273×273×600 μm^3, and number of averages (NA) = 22, total scanning time was around 40 hours.

Fig. (**1**) illustrates the anatomical images, and the maps of scalar diffusion indices, the images show both high SNR and contrast-to-noise ratio (CNR).

2.3. FA Weighted Color Map and Fiber Orientation Map

FA weighted color map and fiber orientation map were used to visually display the water diffusivity in tissue. FA weighted color map was generated by combining the primary eigenvector and FA into red-green-blue color images. The color represents the orientation of tissue alignment: red color represents right–left (or left-right), green indicates anterior–posterior (or posterior-anterior), and blue is assigned to superior–inferior (or inferior–superior). The intensity is proportional to FA, representing the degree of water diffusion anisotropy; the brighter the image, the more consistent alignment there is.

In addition, the major eigenvector (corresponding to the largest eigenvalue) within a voxel is supposed to parallel to the local fiber orientation, so local fiber orientation can be described by visualizing the diffusion ellipsoid with three semi-axis length λ_1, λ_2, and λ_3, along three eigenvector directions $\mathbf{v}_1, \mathbf{v}_2$, and $\mathbf{v}_3$ respectively.

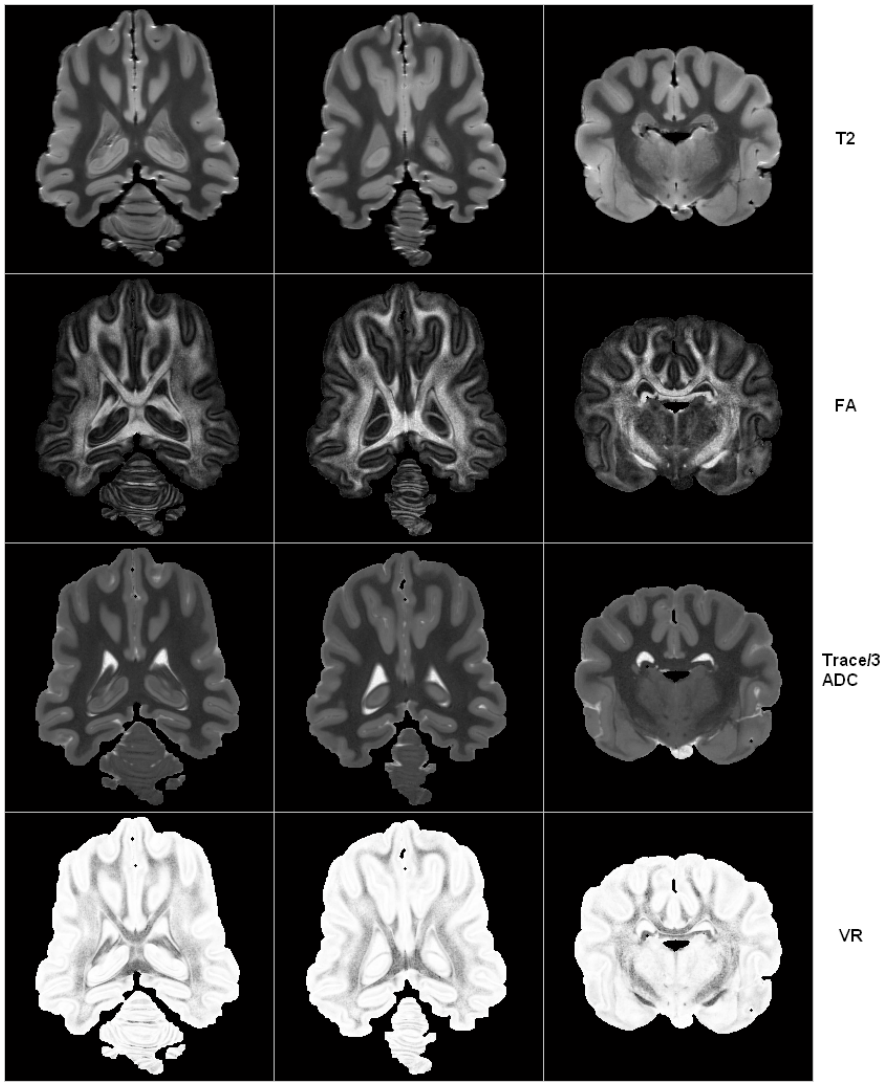

Fig. (1). Three representative slices from the formalin-fixed dog brains. The 1st and 2nd columns are in coronal direction, the 3rd column is axial slice. From the 1st to 4th rows, the T2 weighted images, FA maps, Trace/3 ADC maps, and VR maps are shown respectively.

2.4. Quantitative Analysis

DTI indices (FA, Trace/3 ADC, and VR) were calculated as mean ± SD (standard deviation) within the brain ROIs. These ROIs were selected from seven distinct brain regions: internal capsule, corpus callosum, caudate nucleus, hippocampus, thalamus, fornix, and cerebral cortex, based on the canine brain atlas from the College of Veterinary Medicine, University of Minnesota (http://vanat.cvm.umn.-edu/brainsect/) and [20].

3. RESULTS

3.1. Visual Displays of Diffusion Anisotropy

The improvement in image quality and spatial resolution, with a high field system, multiple-angular diffusion encoding scheme, spin-echo diffusion imaging sequence, and optimized parameters, allows excellent visualization of very small fiber tracts related to microanatomies. Fig. (**2**) shows the FA weighted color maps as well as the fiber orientation map within the selected ROI where the details of the hippocampus region are shown clearly.

3.2. Comparisons of Diffusion Indices

Comparisons of diffusion indices from the selected ROIs are demonstrated in Fig. (**3**), these quantitative diffusion values are also listed in Table **1** for clarity. As expected, FA values in the corpus callosum, fornix, and internal capsule are much higher (ranging from 0.45 to 0.64) than those from the caudate nucleus, hippocampus, thalamus, and cerebral cortex (ranging from 0.14 to 0.20). VR has an inverse conclusion to FA, but the general value of VR is larger than FA. Compared to FA and VR, the change of Trace/3 ADC is smaller between ROIs.

4. DISCUSSION AND CONCLUSION

In this paper, fixed dog brains were examined using high resolution DTI experiments at 7T encoded with 25 directions, the experiment shows much higher scalar contrast as well as more accurate fiber structures of both major and minor tissue structures. A series of quantitative and visual analyses were performed to assess the brain microanatomy using scalar diffusion index, FA weighted color map and fiber orientation map.

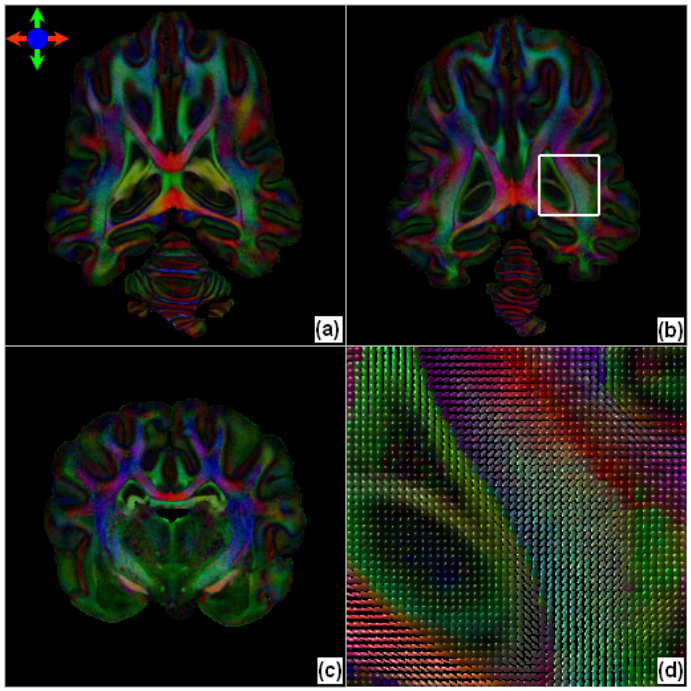

Fig. (2). (**a**) - (**c**): FA weighted color maps of three slices in dog brain. The color represents the orientation of tissue alignment: red color represents right-left (or left-right), green indicates anterior–posterior (or posterior–anterior), and blue is assigned to superior-inferior (or inferior-superior). The intensity is proportional to FA value, representing the degree of water diffusion anisotropy; (**d**) is the fiber orientation map of the ROI in (**b**), in which the local orientations of tissues are visualized clearly.

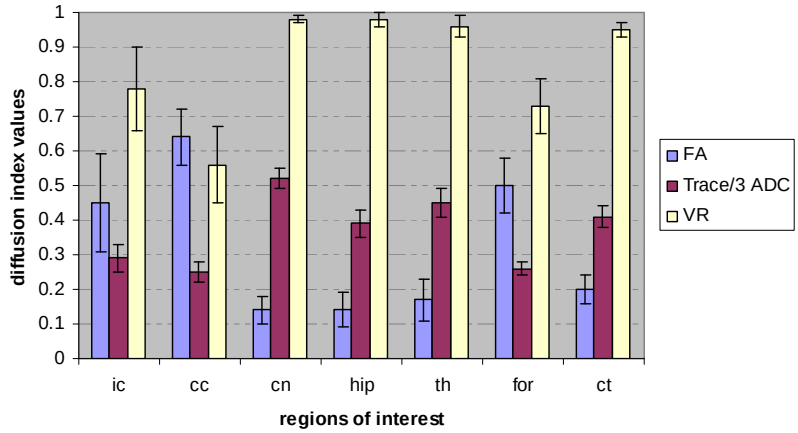

Fig. (3). Diffusion indices within the selected ROIs: internal capsule (ic), corpus callosum (cc), caudate nucleus (cn), hippocampus (hip), thalamus (th), fornix (for), and cerebral cortex (ct).

Table 1. Quantitative Measurements of Diffusion Indices

ROI / Diffusion Index	ic	cc	cn	hip	th	for	ct
FA	0.45±0.14	0.64±0.08	0.14±0.04	0.14±0.05	0.17±0.06	0.50±0.08	0.20±0.04
Trace/3 ADC ($\times 10^{-3}$ mm^2/s)	0.29±0.04	0.25±0.03	0.52±0.03	0.39±0.04	0.45±0.04	0.26±0.02	0.41±0.03
VR	0.78±0.12	0.56±0.11	0.98±0.01	0.98±0.02	0.96±0.03	0.73±0.08	0.95±0.02

Abbreviations: ic - internal capsule, cc - corpus callosum, cn - caudate nucleus, hip - hippocampus, th - thalamus, for - fornix, ct - cerebral cortex.

Quantitative analysis of diffusion indices (FA, Trace/3 ADC, and VR) was performed on seven representative ROIs: internal capsule, corpus callosum, caudate nucleus, hippocampus, thalamus, fornix, and cerebral cortex.

FA weighted color map and fiber orientation map show extremely explicit tissue interfaces as well as the local diffusion orientations (fiber orientations) in the brain areas, which resulted from the better estimation of diffusion tensor model and the excellent contrasts between highly organized and less organized tissue structures.

In summary, this paper provides an accurate and yet quantitative imaging method to map the microstructure in fixed dog brains with high angular DTI at 7T. Using this procedure, high resolution and high SNR DTI images can be obtained, which makes it more accurate to detect both the major and minor fiber tracts. These verify that the method is very useful to study the microstructure in dog brains. To our knowledge, systematic and quantitative DTI examination for dog brain has not been reported previously, the methodology can also be used to study the functional microanatomy of other animal models.

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